Natera, Inc. v. NeoGenomics Laboratories, Inc.

C.A. No. 1:23-cv-629-CCE-JLW

***Markman* Hearing**

March 11, 2025

natera | quinn emanuel trial lawyers
quinn emanuel urquhart & sullivan, llp

Four Disputed Terms

1. A method for preparing biological samples useful for monitoring the progression of cancer in a subject, the method comprising:

(a) performing sequencing on a tumor biopsy sample of the subject to identify a plurality of tumor-specific mutations, wherein the tumor-specific mutations comprise one or more single nucleotide variant (SNV) mutations;

(b) evaluating results of the sequencing on the tumor biopsy sample to determine a plurality of target loci specific to the subject, wherein each target locus spans a tumor-specific mutation of the identified plurality of tumor-specific mutations; and

(c) assaying cell-free DNA isolated from a plurality of biological samples obtained from the subject at different time points, wherein the assaying comprises:

performing targeted multiplex PCR amplification to amplify the plurality of target loci together in the same reaction volume from the isolated cell-free DNA using primers specific to the plurality of target loci for the individual subject; and (A)

C performing high-throughput sequencing of the amplified DNA comprising the plurality of target loci to obtain sequence reads, wherein an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA having the SNV locus is detected from the sequence reads. (B) (D)

'596 Patent, Claim 1.

A. “the isolated cell-free DNA”

’596 Patent, Claim 1

"the isolated cell-free DNA"

Natera's Proposal	NeoGenomics' Proposal
Plain and ordinary meaning, which includes cell-free DNA isolated from any of the plurality of biological samples obtained from the subject at different time points.	"Plain and ordinary" meaning, which is cell-free DNA isolated from a plurality of biological samples obtained from the subject and excludes cell-free DNA isolated from a single biological sample obtained from the subject.

- Claim recites "at different timepoints"
- Claim does not recite combining samples

- Reads out "at different timepoints"
- Reads sample pooling and simultaneous analysis limitation into the claim

Doc. 372 at 4-5; Doc. 373 at 12-13; Doc. 378 at 3-4; Doc. 380 at 2-3.

Parties Dispute Antecedent Basis Of "the isolated cell-free DNA"

- Natera: "cell-free DNA isolated from a plurality of biological samples obtained from the subject *at different time points*"
- NeoGenomics: "cell-free DNA isolated from a plurality of biological samples obtained from the subject"

What is claimed is:

1. A method for preparing biological samples useful for monitoring the progression of cancer in a subject, the method comprising:

(a) performing sequencing on a tumor biopsy sample of the subject to identify a plurality of tumor-specific mutations, wherein the tumor-specific mutations comprise one or more single nucleotide variant (SNV) mutations;

(b) evaluating results of the sequencing on the tumor biopsy sample to determine a plurality of target loci specific to the subject, wherein each target locus spans a tumor-specific mutation of the identified plurality of tumor-specific mutations; and

(c) assaying cell-free DNA isolated from a plurality of biological samples obtained from the subject ~~at different time points,~~ wherein the assaying comprises:

performing targeted multiplex PCR amplification to amplify the plurality of target loci together in the same reaction volume from the isolated cell-free DNA using primers specific to the plurality of target loci for the individual subject; and

performing high-throughput sequencing of the amplified DNA comprising the plurality of target loci to obtain sequence reads, wherein an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA having the SNV locus is detected from the sequence reads.

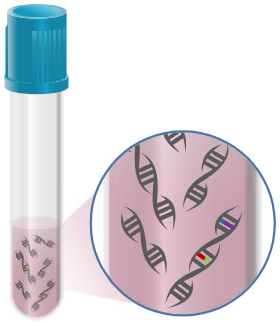
NeoGenomics

'596 Patent, Claim 1.

Doc. 372 at 4;
Doc. 373 at 11;
Doc. 378 at 2-3;
Doc. 380 at 2.

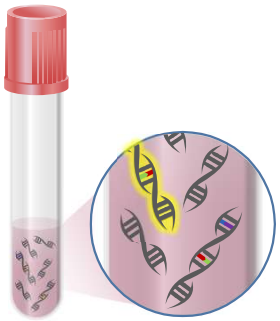
Claim Recites Preparing Samples Useful For Monitoring Cancer Progression

Timepoint 1



Targeted multiplex PCR amplification

Timepoint 2

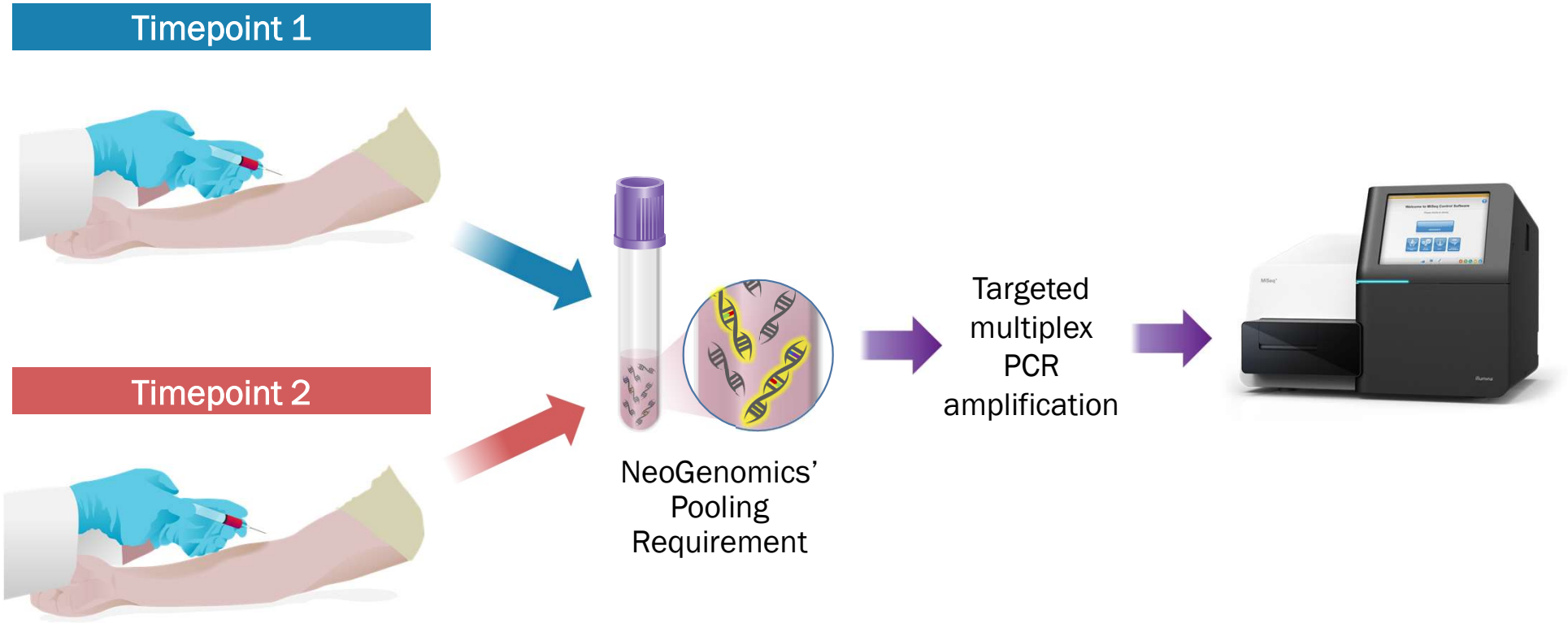


Targeted multiplex PCR amplification

Doc. 372 at 6-8; Doc. 378 at 4.

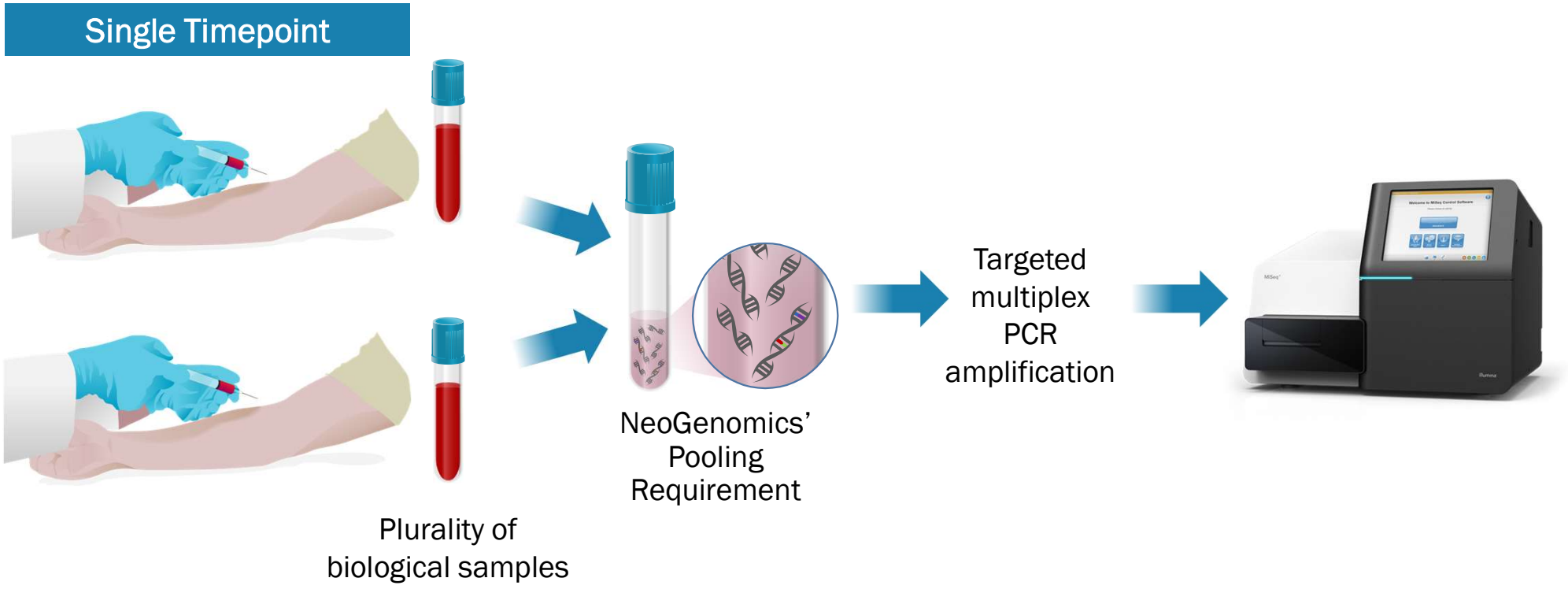
NeoGenomics' Construction Ignores Monitoring Cancer Progression

- *Monitoring progression* means assaying different samples over time
- NeoGenomics' construction would combine samples and assay only once



Doc. 372 at 6-8; Doc. 373 at 12-13; Doc. 378 at 4-5; Doc. 380 at 2-3.

NeoGenomics' Sample Pooling Construction Ignores Timepoint Limitation



Doc. 380 at 1, 5-6.

NeoGenomics Misdirects From Disclosure Of Repeat Testing

What NeoGenomics Ignores:

vidual (such as on the same or a different sample). In some embodiments, a subject diagnosed with a disease or disorder (such as cancer) undergoes repeat testing using a method of the invention or known testing for the disease or disorder at multiple time points to monitor the progression of the disease or disorder or the remission or reoccurrence of the disease or disorder.

Doc. 352-10 ('596 Patent) at 27:48-54; *see also id*. at 140:59-63.

- NeoGenomics focuses on combining different sample types (not recited) and ignores repeat testing over time (as recited)

Doc. 372 at 9-10; Doc. 378 at 6.

NeoGenomics' Misguided Focus:

In some embodiments, the database includes records with any of the following information for one or more subjects: any polymorphisms/mutations identified, any known association of the polymorphisms/mutations with cancer or an increased risk for cancer, effect of the polymorphisms/mutations on the expression or activity level of the encoded mRNA or protein, fraction of cancerous DNA, RNA or cells out of the total DNA, RNA, or cells in sample, source of sample used to identify the polymorphisms/mutations (such as a blood sample or sample from a particular tissue), number of cancerous cells, size of tumor(s), results from later repeating the test (such as repeating the test to monitor the progression or remission of the cancer), results of other tests for cancer, type of cancer the subject was diagnosed with, treatment(s) administered, response to such treatment(s), side-effects of such treatment(s), symptoms (such as symptoms associated with cancer), length and number of remissions, length of survival (such as length of time from initial test until death or length of time from cancer diagnosis until death), cause of death, and combinations thereof. In some embodiments, the response to treat-

Doc. 352-10 ('596 Patent) at 139:35-47.

NeoGenomics' "Sample Pooling" Relies On Unclaimed Embodiments

In some embodiments, the sample includes a single cell or includes DNA and/or RNA from a single cell. In some embodiments, multiple individual cells (e.g., at least 5, 10, 20, 30, 40, or 50 cells from the same subject or from different subjects) are analyzed in parallel. In some embodiments, cells from multiple samples from the same individual are combined, which reduces the amount of work compared to analyzing the samples separately. Combining multiple samples can also allow multiple tissues to be tested for cancer simultaneously (which can be used to provide or more thorough screening for cancer or to determine whether cancer may have metastasized to other tissues).

Doc. 352-10 ('596 Patent) at 95:27-38.

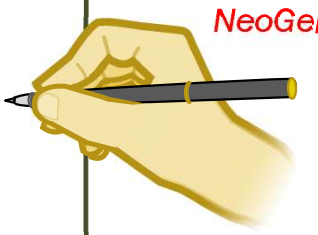
- No mention of cell-free DNA
- No mention of monitoring cancer progression
- NeoGenomics fails to reconcile its "sample pooling" argument with claim's recited "different time points"

Doc. 373 at 12-13;
Doc. 378 at 5-6;
Doc. 380 at 3.

NeoGenomics Rewrites The Claims

that is first pooled from multiple biological samples taken from the subject

(c) assaying cell-free DNA isolated from a plurality of biological samples obtained from the subject ~~at different time points~~, wherein the assaying comprises:
performing targeted multiplex PCR amplification to amplify the plurality of target loci together in the same reaction volume from the isolated cell-free DNA using primers specific to the plurality of target loci for the individual subject; and



Doc. 352-10 ('596 Patent), Claim 1.

- NeoGenomics writes in language based on erroneous antecedent basis
- Pooling is not a requirement of the claim

Doc. 372 at 4-5; Doc. 373 at 12-13;
Doc. 378 at 3-4; Doc. 380 at 2-3.

Eastman and *Innovative* Are Inapposite

Eastman court held:

- "The salt" referred to the only prior mention of "salt" in the claim.

Eastman Chem. Co. v. Aktiengesellschaft, 47 F. App'x 566, 573 (Fed. Cir. 2002).

Innovative court held:

- Courts should consider "the claim in full" when determining "the antecedent basis for each element."

Innovative Memory Sys., Inc. v. Micron Tech., Inc., 781 F. App'x 1013, 1017 (Fed. Cir. 2019).

Here:

- NeoGenomics' construction ignores the "at different points in time" limitation.

Doc. 378 at 4; Doc. 373 at 12-13.

Guardant Is Also Inapposite

Guardant Claim Language:

> 1. A method for quantifying single nucleotide variant tumor markers in cell-free DNA from a subject, comprising:
>
> . . .
>
> (d) sequencing the amplified non-uniquely tagged progeny polynucleotides to produce ***a plurality of sequence reads*** from each parent polynucleotide, . . .
>
> (e) grouping ***the plurality of sequence reads*** produced from each non-uniquely tagged parent polynucleotide into families[.]

Guardant Health, Inc. v. Found. Med., Inc., Nos. 17-1616-LPS-CJB, 17-1623-LPS-CJB, 2019 WL 5677748, at *10 (D. Del. Nov. 1, 2019).

Guardant court held:

- All of "the plurality of sequence reads" required grouping
- Consistent with the entire antecedent phrase

Here:

- NeoGenomics omits part of the antecedent phrase re: timepoints
- "isolated cell-free DNA" term does not recite "plurality"

Doc. 380 at 3-4.

B. **"performing high-throughput sequencing of the amplified DNA comprising the plurality of target loci to obtain sequence reads, wherein an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA having the SNV locus is detected from the sequence reads"**

'596 Patent, Claim 1

"performing high-throughput sequencing…from the sequence reads"

What is claimed is:

1. A method for preparing biological samples useful for monitoring the progression of cancer in a subject, the method comprising:

(a) performing sequencing on a tumor biopsy sample of the subject to identify a plurality of tumor-specific mutations, wherein the tumor-specific mutations comprise one or more single nucleotide variant (SNV) mutations;

(b) evaluating results of the sequencing on the tumor biopsy sample to determine a plurality of target loci specific to the subject, wherein each target locus spans a tumor-specific mutation of the identified plurality of tumor-specific mutations; and

(c) assaying cell-free DNA isolated from a plurality of biological samples obtained from the subject at different time points, wherein the assaying comprises:

performing targeted multiplex PCR amplification to amplify the plurality of target loci together in the same reaction volume from the isolated cell-free DNA using primers specific to the plurality of target loci for the individual subject; and

performing high-throughput sequencing of the amplified DNA comprising the plurality of target loci to obtain sequence reads, wherein an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA having the SNV locus is detected from the sequence reads.

Doc. 352-10 ('596 Patent), Claim 1.

- The entire step should be construed
- All parts of the step – including to obtain sequence reads – inform a POSA's understanding of what "is detected" means

Doc. 372 at 10-14;
Doc. 373 at 13-14;
Doc. 378 at 6-12;
Doc. 380 at 6-7.

"performing high-throughput sequencing…from the sequence reads"

> performing high-throughput sequencing of the amplified DNA comprising the plurality of target loci to obtain sequence reads, wherein an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA having the SNV locus is detected from the sequence reads.

Doc. 352-10 ('596 Patent), Claim 1.

Natera's Proposal

Plain and ordinary meaning, which means that the terms are part of the "performing high-throughput sequencing . . . to obtain sequence reads" step.

- "is detected" happens during a single sequencing step to obtain sequence reads

NeoGenomics' Proposal

"Plain and ordinary" meaning, which means that only two sub-terms should be construed.

- Reads out "comprising…to obtain sequence reads" language
- Ignores "wherein…" language

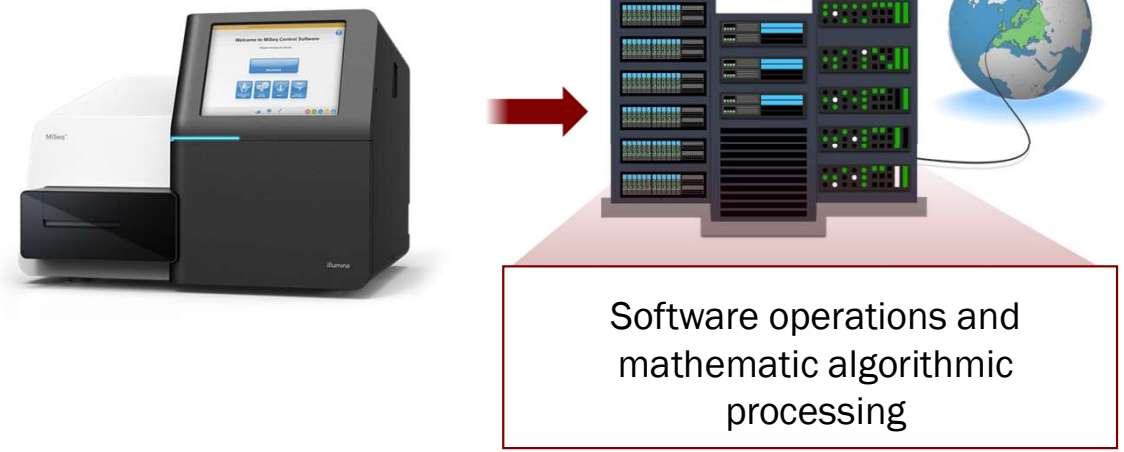
Doc. 372 at 10-14; Doc. 373 at 13-14; Doc. 378 at 6-12; Doc. 380 at 6-7.

The Real Dispute Is Over Where, When, And How The SNV Is Detected

Natera:

- Detecting happens in sequencer, during sequencing, with fluorescent signals

NeoGenomics:



- Detecting happens in an overseas computer server, after sequencing, using an algorithm

Doc. 372 at 10-14; Doc. 373 at 13-14; Doc. 378 at 6-12; Doc. 380 at 6-7.

NeoGenomics' Construction Is A Moving Target

NeoGenomics' Opening Brief:

Plain and ordinary meaning, which is wherein an SNV mutation is determined to be present from the sequence reads.

Doc. 373 at 14.

performing high-throughput sequencing of the amplified DNA comprising the plurality of target loci to obtain sequence reads, wherein an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA having the SNV locus ~~is detected~~ from the sequence reads.

Doc. 352-10 ('596 Patent), Claim 1.

NeoGenomics' Responsive Brief:

The claim language, specification, and prosecution history all show that the plain and ordinary meaning of "an SNV mutation … is detected from the sequence reads" is that the sequence reads are analyzed to determine whether the SNV is present. The claim language states that high-throughput sequencing is performed "to obtain sequence reads"

Doc. 380 at 8.

performing high-throughput sequencing of the amplified DNA comprising the plurality of target loci to obtain sequence reads, wherein ~~an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA having the SNV locus is detected from the sequence reads~~.

Doc. 352-10 ('596 Patent), Claim 1.

Claim Terms Should Be Construed Consistently Across Patents

’454 Patent, Claim 1:

> sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads, wherein the sequencing has a depth of read of at least 50,000 per target locus.

Doc. 1-1 (’454 Patent), Claim 1.

’596 Patent, Claim 1:

> performing high-throughput sequencing of the amplified DNA comprising the plurality of target loci to obtain sequence reads, wherein an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA having the SNV locus is detected from the sequence reads.

Doc. 352-10 (’596 Patent), Claim 1.

Court’s Construction:

> The structure of Claim 1 of the ‘454 patent indicates that the sequencing and detecting are all part of one sequencing step. Three discrete steps of Claim 1, the steps
>
> * * *
>
> The Court adopts Natera’s construction.

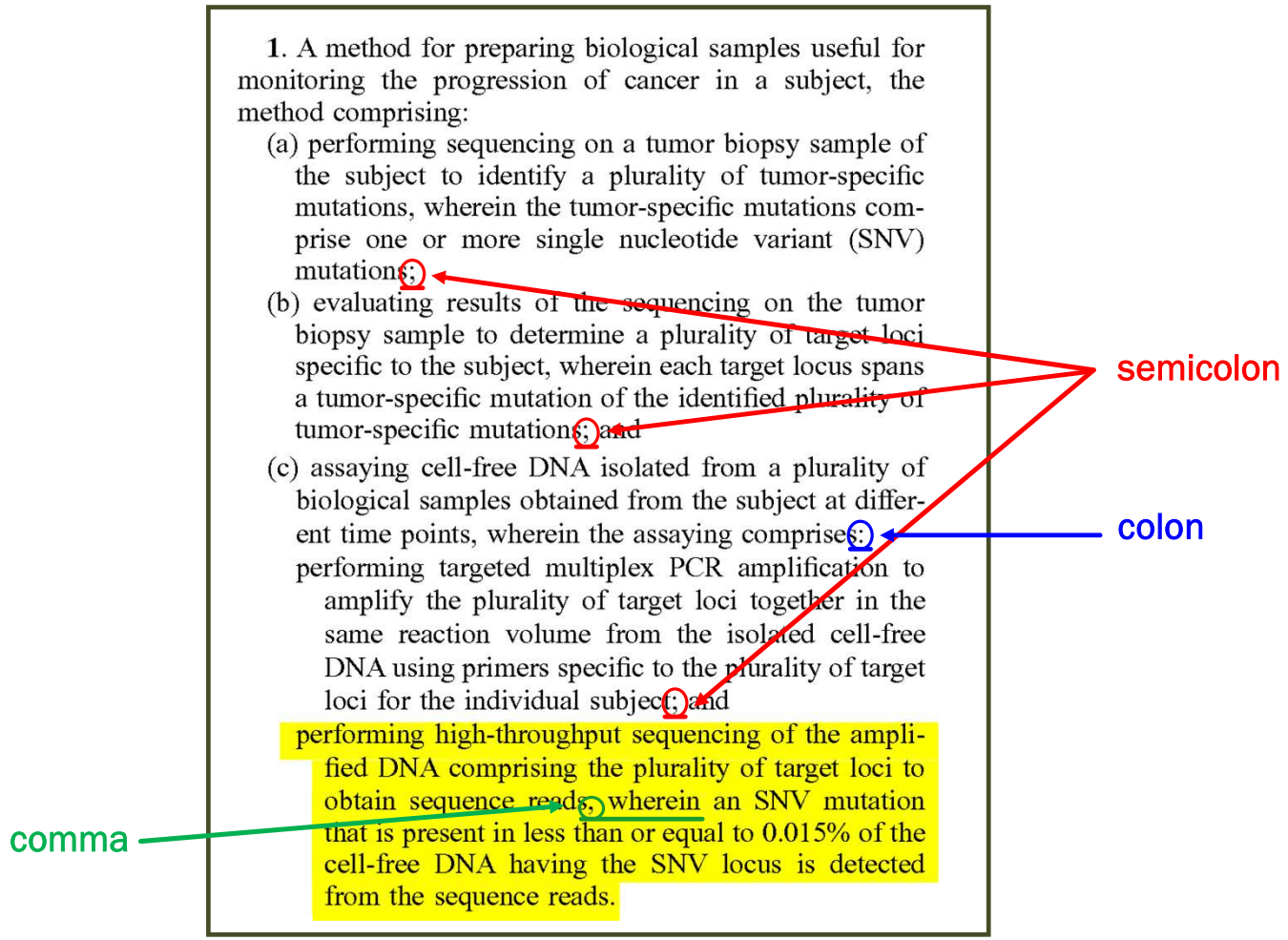
Doc. 280.

- Court should construe ’596 Patent similarly: detecting SNVs from the sequence reads occurs during sequencing to obtain sequence reads

B.1 The Claim Must Be Read According To English Grammar

’596 Patent, Claim 1

"Performing...from the sequence reads" Refers To A Single Step



1. A method for preparing biological samples useful for monitoring the progression of cancer in a subject, the method comprising:

(a) performing sequencing on a tumor biopsy sample of the subject to identify a plurality of tumor-specific mutations, wherein the tumor-specific mutations comprise one or more single nucleotide variant (SNV) mutations;

(b) evaluating results of the sequencing on the tumor biopsy sample to determine a plurality of target loci specific to the subject, wherein each target locus spans a tumor-specific mutation of the identified plurality of tumor-specific mutations; and

(c) assaying cell-free DNA isolated from a plurality of biological samples obtained from the subject at different time points, wherein the assaying comprises:

performing targeted multiplex PCR amplification to amplify the plurality of target loci together in the same reaction volume from the isolated cell-free DNA using primers specific to the plurality of target loci for the individual subject; and

performing high-throughput sequencing of the amplified DNA comprising the plurality of target loci to obtain sequence reads, wherein an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA having the SNV locus is detected from the sequence reads.

Doc. 352-10 ('596 Patent), Claim 1.

Doc. 372 at 10-14;
Doc. 378 at 6-12.

Dr. Lennon: Punctuation And Grammar Important For Construction

Dr. Niall Lennon
NeoGenomics' Expert

Q. When you're looking at the language of the claim, does grammar inform your interpretation of what is meant by the claim?

A. So I guess if you could be more specific in terms of -- grammar informs how I read it, but I'm not a grammarian, so I don't know if I'm applying some special type of grammar.

Q. What do you mean by a "special type of grammar"?

A. It's my understanding from this kind of work that there's -- that people focus a lot on the placement of colons, semicolons, commas, periods. That's not my area of expertise, but I understand that it can be important.

Doc. 378 at 8-9; Doc. 378-4 at 19:11-20:20.

B.2 "wherein an SNV mutation . . . is detected from the sequence reads"

'596 Patent, Claim 1

"wherein an SNV mutation...is detected from the sequence reads"

Natera's Proposal	NeoGenomics' Proposal
Plain and ordinary meaning, which means that the "wherein" clause informs the mechanics of how the high-throughput sequencing is performed.	Plain and ordinary meaning, which is wherein an SNV mutation is determined to be present from the sequence reads.

- Sequencing and detecting occur inside the sequencer
- NeoGenomics' technical expert and multiple corporate witnesses agree with Natera

- Rewrites "wherein" clause
- Reads in data processing limitation *after* sequencing
- Misunderstands the science

Doc. 373 at 19-20; Doc. 378 at 16, 18-20; Doc. 379, ¶¶ 26-37, 67.

Preferred Embodiments Use Illumina Sequencers

ments, PCR amplification is used to amplify target loci. In some embodiments, the amplified DNA is sequenced (such as sequencing using an ILLUMINA IIGAX or HiSeq sequencer). In some embodiments, the amplified DNA is

Doc. 352-10 ('596 Patent) at 96:37-40; *see also id*. at 58:32-45, 62:1-13, 152:61-67, 164:7-14.

Doc. 373 at 14-15;
Doc. 380 at 14.

Illumina Specifications Explain Detecting In Sequencer

System Specification Sheet: Sequencing

illumina

HiSeq® 2500 Sequencing System

Unsurpassed power and efficiency for production scale sequencing.

HiSeq System Highlights

- Incredible Speed and Throughput
  Complete projects in record time with the highest daily throughput of any individually sold sequencing system
- Unprecedented Flexibility
  Choose between rapid-run and high-output modes, offering scalable output from 10 Gb to 1 Tb for a broad range of applications and sample sizes
- Exceptional Data Quality
  Build confidence in your results with the most widely adopted and proven Illumina SBS chemistry
- Complete End-to-End Sequencing Solutions
  Enjoy integrated streamlined workflows from library prep through data analysis

Introduction

The HiSeq 2500 has become the undisputed powerhouse of Illumina sequencing systems for highthroughput sequencing applications (Figure 1). To date, millions of samples have been sequenced and thousands of papers have been published utilizing Illumina sequencing technology. With unrivaled data quality, flexible run configurations, and a 10 gigabase (Gb) to 1 terabase (Tb) per run capacity, the HiSeq 2500 is the production platform of choice for all major genome centers and leading institutions around the world.1

Incredible Speed and Throughput

The HiSeq 2500 System delivers the highest daily throughput and total yield of any individually sold sequencer on the market today. With a massive daily throughput of 160 Gb per day or 1 Tb per run, more samples can be simultaneously sequenced at a greater depth than ever before—producing richer, more meaningful data. Large studies can be completed with reduced hands-on time and reagent cost. In high output mode, the HiSeq 2500 System can process 8 human genomes at 30× coverage or 150 human exomes, assuming 4 Gb per exome at 2 × 75 bp, per run. The HiSeq 2500 System, in high-output mode, is perfect for production scale projects with large genome sizes or studies with hundreds of samples.

Unprecedented Flexibility

While the HiSeq 2500 System can generate massive data, it is also highly flexible. The HiSeq 2500 is the first Illumina sequencing platform to feature 2 run modes—rapid run and high-output run mode. Furthermore, the system can be configured to run 1 flow cell alone or 2 flow cells simultaneously—enabling a scalable solution tailored to your specific research needs (Figure 2). Rapid-run mode leverages on-board cluster generation and reduced cycle times to significantly

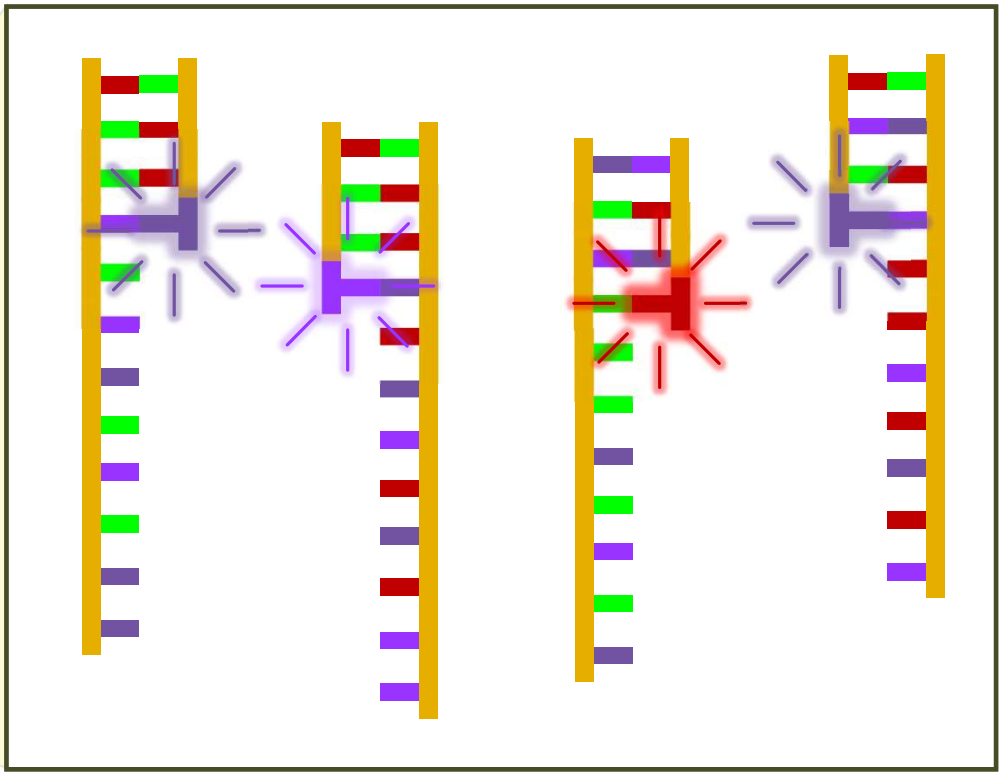
With the HiSeq 2500 System, high data quality is ensured by leveraging the most widely adopted chemistry in the industry: sequencing by synthesis (SBS). SBS technology on the HiSeq 2500 utilizes a proprietary reversible terminator-based method that detects single bases as they are incorporated into DNA template strands.

- Base calls are made as the read is generated by detecting the base's fluorescence

Doc. 372-2 at 1; Doc. 352-10 ('596 Patent) at pp.24.
Doc. 373 at 14-15;
Doc. 380 at 14.

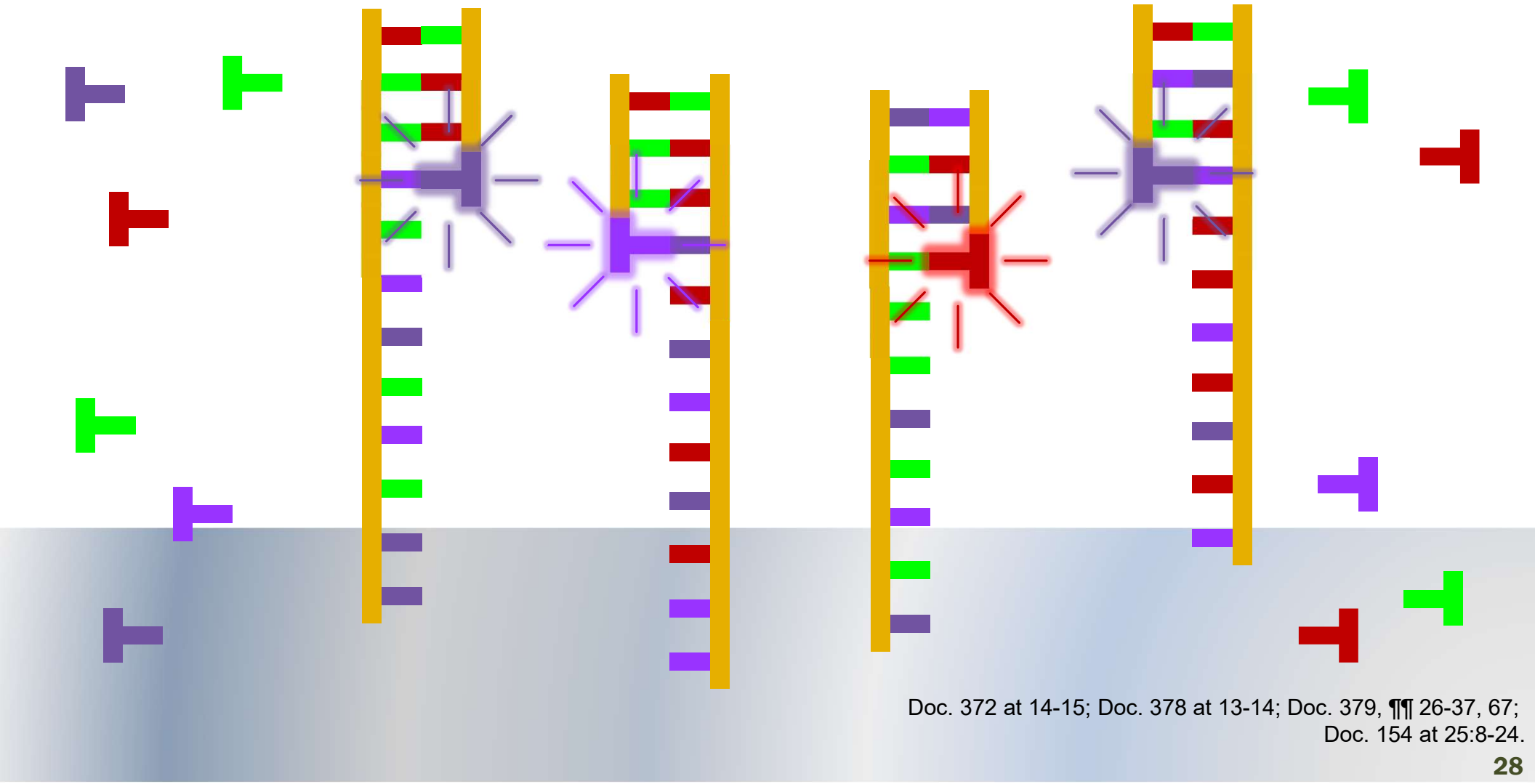
Detection Occurs When Sequencer Reads The Sequence

Next-generation Sequencer



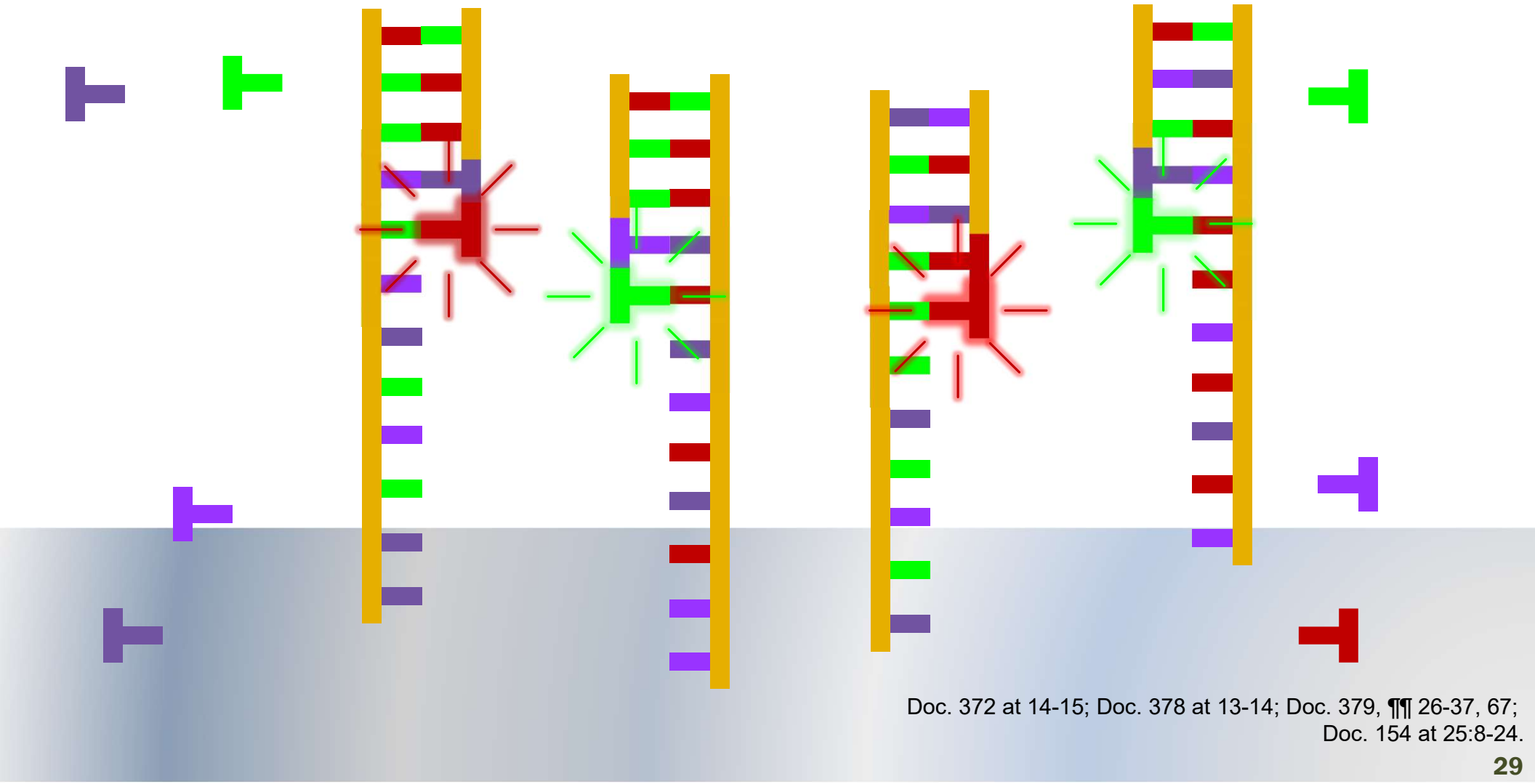
Doc. 372 at 14-15; Doc. 378 at 13-14; Doc. 379, ¶67; Doc. 154 at 25:8-24.

Sequencing With Fluorescent Nucleotides



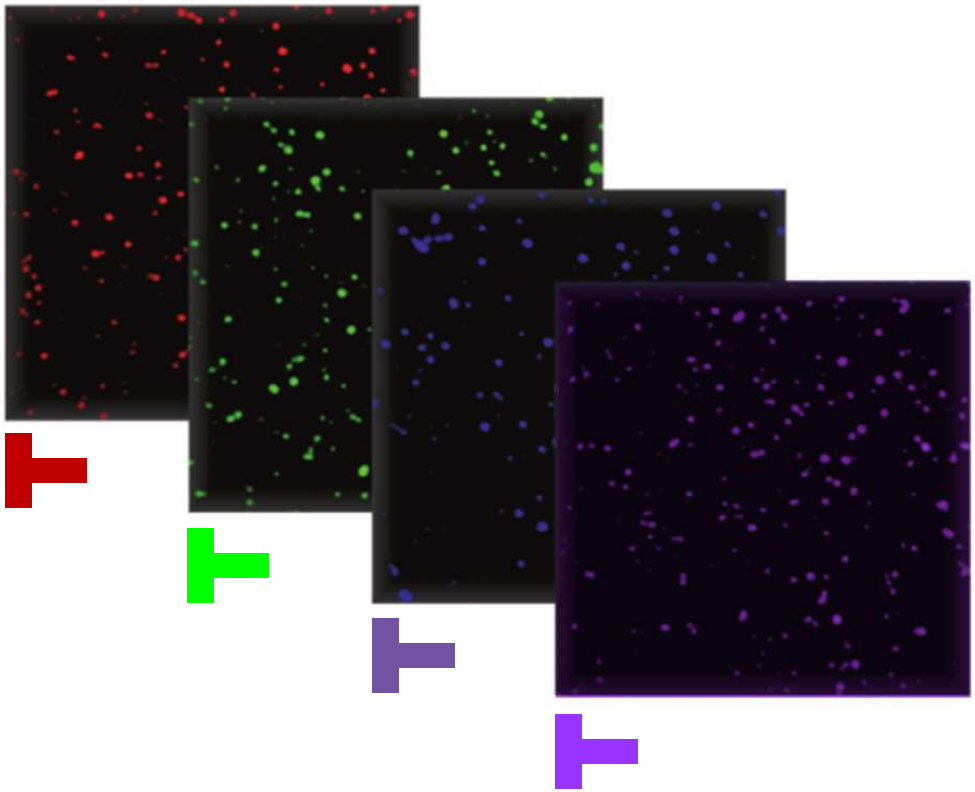
Doc. 372 at 14-15; Doc. 378 at 13-14; Doc. 379, ¶¶ 26-37, 67; Doc. 154 at 25:8-24.

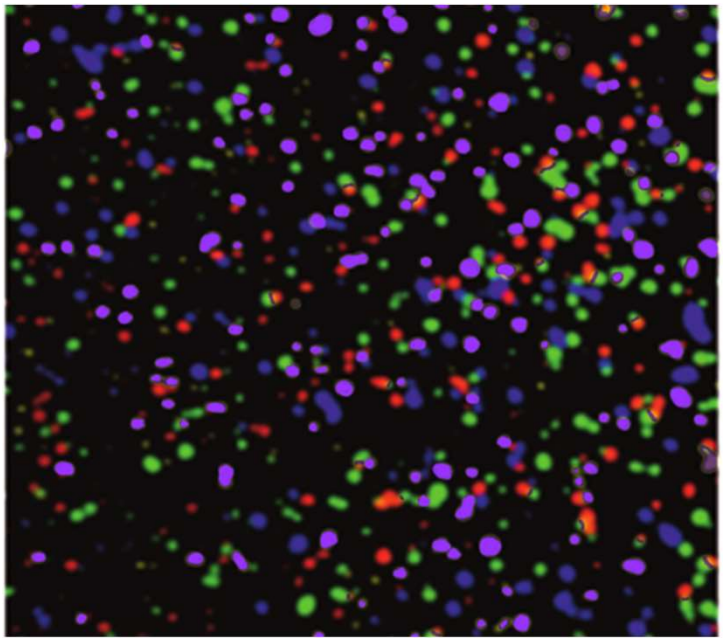
Sequencing With Fluorescent Nucleotides



Doc. 372 at 14-15; Doc. 378 at 13-14; Doc. 379, ¶¶ 26-37, 67; Doc. 154 at 25:8-24.

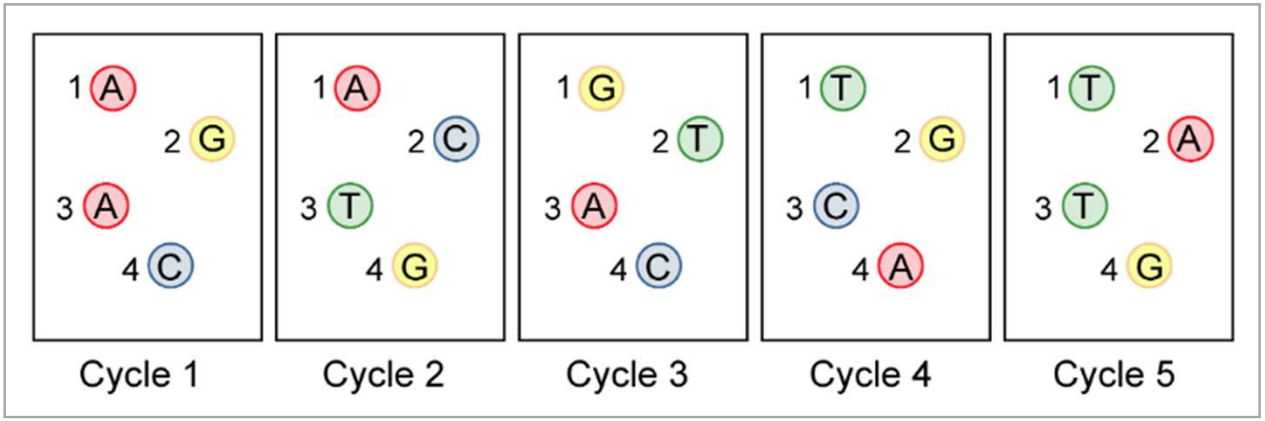
Sequence Reads: Imaging At Each Step Captures Identity Of Bases Added

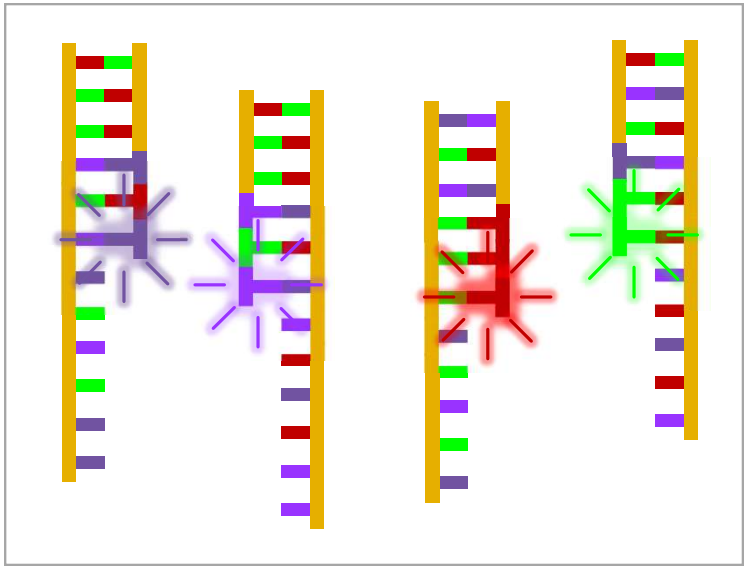




Doc. 372 at 14-15; Doc. 378 at 13-14; Doc. 379, ¶¶ 26-37, 67; Doc. 154 (Technology Tutorial Tr.) at 25:8-24.

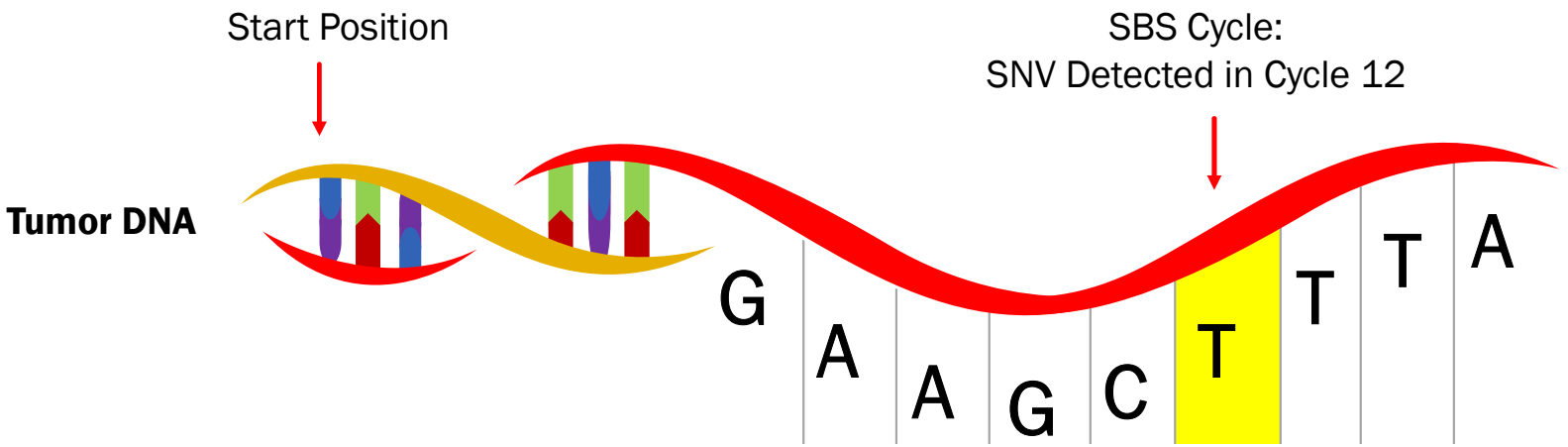
Sequence Reads Are Generated Iteratively In Each Sequencing Cycle





Doc. 372 at 14-15; Doc. 378 at 13-14;
Doc. 379, ¶¶ 26-37, 67;
Doc. 154 (Technology Tutorial Tr.) at 25:8-24.

How SNVs Are Detected In The Sequencing Process

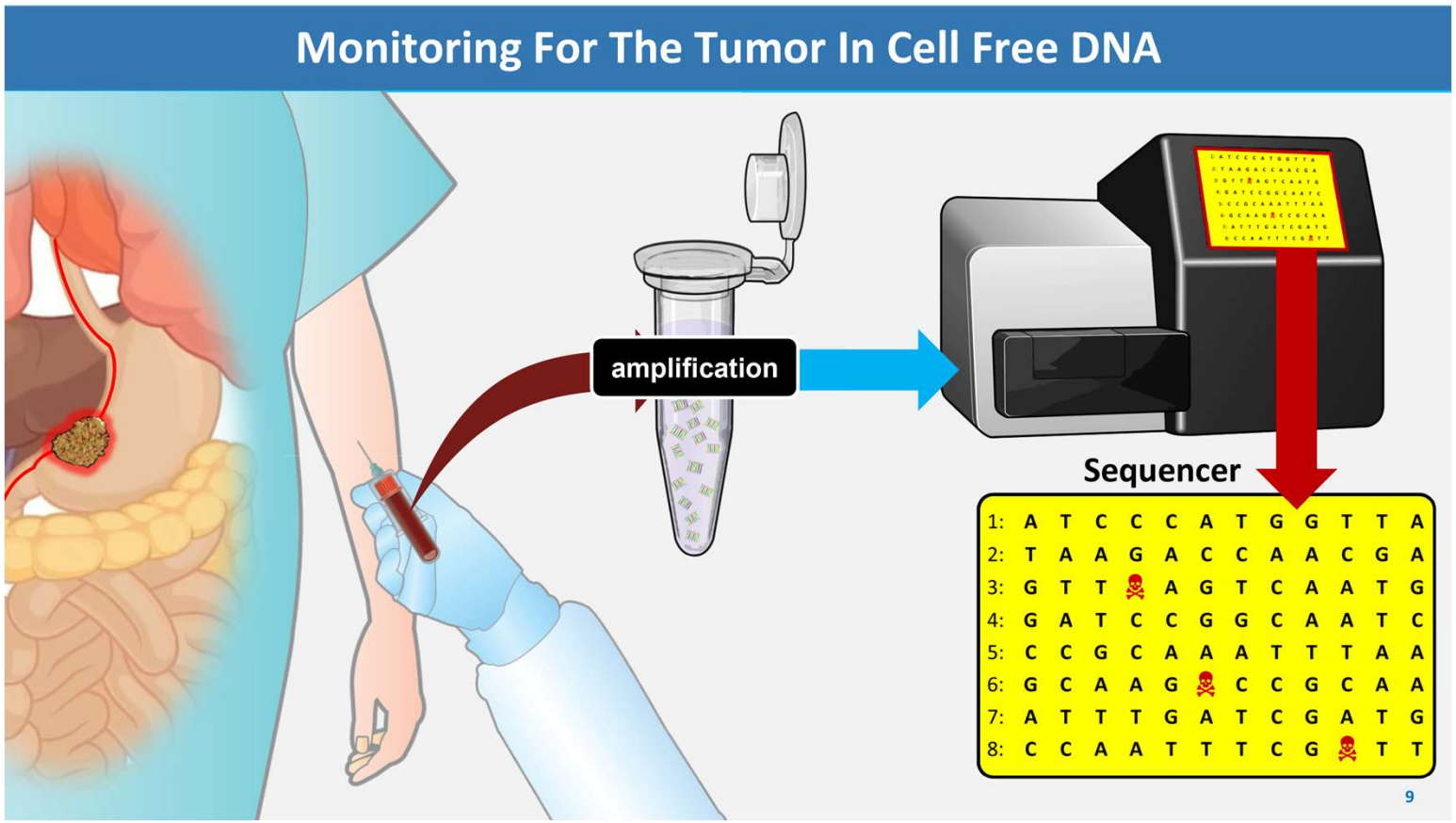


- Amplicons are designed to have SNVs at a certain position
- If SNV is present, its color will be detected by the sequencer

Doc. 378 at 14-15;
Doc. 379, ¶¶ 21-22,35,66,69.

NeoGenomics Agrees With Natera

NeoGenomics Technology Tutorial Slide



NeoGenomics 11/9/2023 Technology Tutorial Slide 9

Dr. Lennon Testified At Trial: Detection Occurs In the Sequencer

Dr. Niall Lennon
NeoGenomics' Expert

Q. Sure, I'll try to help you out. The Read 2 sequencing primer, that primer gets used during the – when it's in the machine, and when it lands, it helps – it actually is part of the process that spits out hey, next one is an A, next one is a G, next one is a T, next one is a C. So it actually -- it's actually a participant in the actual thing where a sequence is getting spit out of the machine; right?

A. Right. We call that the detection step.

Q. So the sequencing step; right?

A. It's where we're doing the interrogation of the colors to understand what bases are there.

Doc. 378 at 18; Doc. 378-1 (Lennon *ArcherDx v. Qiagen* Trial Tr.) at 41:22-42:15.

Dr. Lennon Testified Here: Detection Occurs In the Sequencer

Dr. Niall Lennon
NeoGenomics' Expert

Q. Could you explain to me what occurs as a process of sequencing in the high-throughput sequencer?

A. … And after that incorporation event, a -- there's a laser excitation of the surface of the flow cell which is essentially an optically clear glass slide, and a -- the camera, essentially, takes a photograph or, essentially, you know, detects any emission spectra or any, you know, signal that comes off of those incorporated bases.

Doc. 378 at 18-19; Doc. 378-4 at 23:4-20.

Dr. Forshew: Base Calls / Detection Occurs In the Sequencer

Dr. Tim Forshew
NeoGenomics' Corporative Representative

Q. You would agree that during sequencing using the Illumina sequencers, the sequencer detects a color when a certain base is added to a growing strand, right? The strand that's being sequenced.

A. The sequencer does measure, to my understanding, a most probable color.

Doc. 378 at 20; Doc. 378-5 at ECF 21, 71:12-18.

Mr. Sikri: SNVs Are Detected In the Sequencer

Vishal Sikri
NeoGenomics' Corporative Representative

Q. Okay. A person at NeoGenomics detects an SNV when they review the output from the custom software analysis, is that correct?

A. The SNVs are detected through the sequencer that where the sample is run.

Doc. 378 at 20; Doc. 144-7 at ECF 21, 74:20-21.

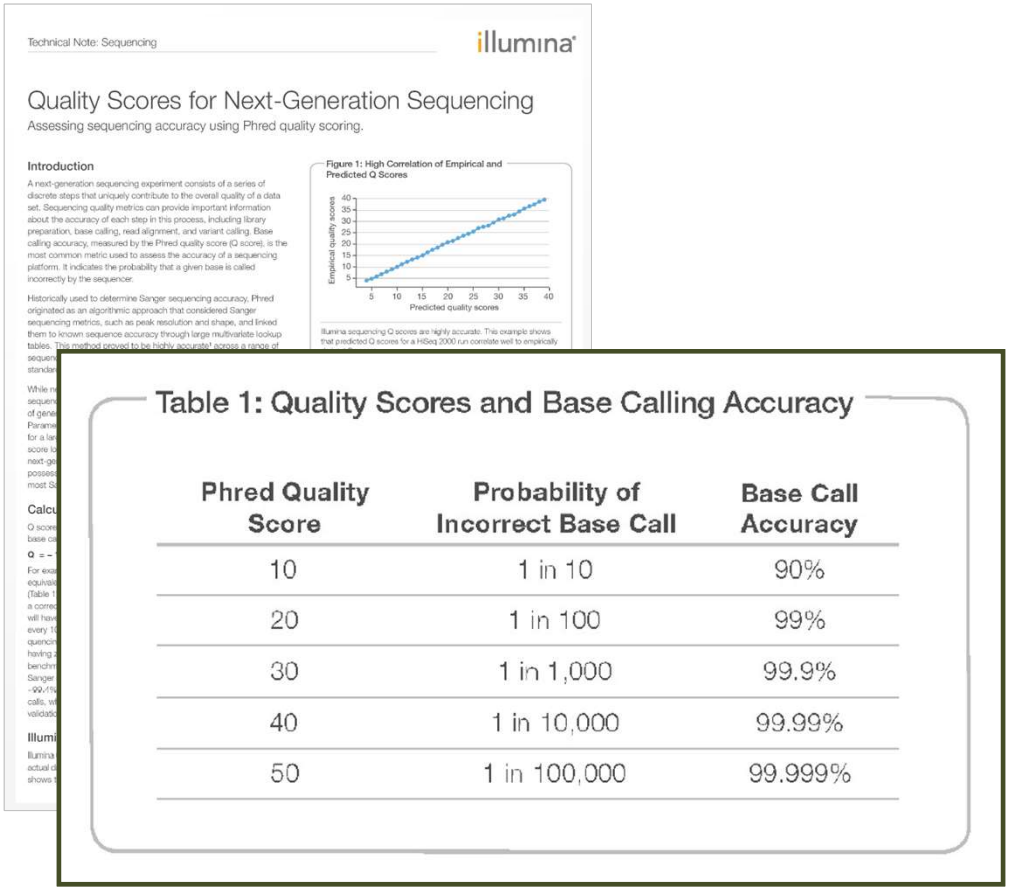
"Sequence Read" Is Defined As Being Generated In A Sequencer

> Sequence Read refers to data representing a sequence of nucleotide bases that were measured, e.g., using a clonal sequencing method. Clonal sequencing may produce sequence data representing single, or clones, or clusters of one original DNA molecule. A sequence read may also have associated quality score at each base position of the sequence indicating the probability that nucleotide has been called correctly.

Doc. 352-10 ('596 Patent) at 31:16-24.

Doc. 378 at 14-15; Doc. 379, ¶¶ 46-47;
Doc. 154 at 25:20-26:5.

Base Calls Made During High-Throughput Sequencing



Technical Note: Sequencing

illumina

Quality Scores for Next-Generation Sequencing

Assessing sequencing accuracy using Phred quality scoring.

Introduction

Figure 1: High Correlation of Empirical and Predicted Q Scores

Table 1: Quality Scores and Base Calling Accuracy

Phred Quality Score	Probability of Incorrect Base Call	Base Call Accuracy
10	1 in 10	90%
20	1 in 100	99%
30	1 in 1,000	99.9%
40	1 in 10,000	99.99%
50	1 in 100,000	99.999%

- Base calls are made one at a time as the read is generated by the sequencer detecting the base's fluorescence
- Quality Score is assigned to every base as a measure of accuracy

Doc. 374-2 at 1-2; Doc. 378 at 14-15; Doc. 379, ¶¶ 38-40,47,67,86.

Dr. Metzker: Illumina Obtains Millions Of Error-Free Sequence Reads

Dr. Michael Metzker
Natera's Expert

Baylor College of Medicine
RICE
REDVAULT BIOSCIENCES
Lasergen

Q. Well, all you need to do is know what the expected position of the SNV is, and look at the sequencing readout for that position, and see if it does or doesn't correspond to the SNV of interest, right? At that point, you've detected the SNV?

A. You absolutely have. And Illumina even tells itself it has millions and millions of error-free sequence reads. So there's many, many sequence reads that are error-free, and you can detect that mutation in that position.

Doc. 388-1 at 89:11-20.

Dr. Lennon: Even With Errors, Sequencing And Detection Is One Step

Dr. Niall Lennon
NeoGenomics' Expert

Dr. Lennon's '596 Patent IPR Declaration:

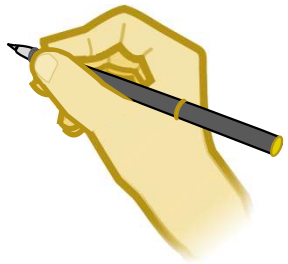
> (Ex.1043, 1.) Because base calls are sometimes incorrect due to sequencing errors, NGS platforms typically also generate quality scores for each sequenced base. (*Id.*) Sequencing platforms that carry out NGS use a variety of sequencing and detection biochemistry but generally have a common, i.e., conceptually similar, workflow to produce these sequence reads. (Ex.1068, 2.)

Doc. 378 at 19; Doc. 378-3, ¶81.

NeoGenomics Rewrites The Claim In Its Opening Brief

performing high-throughput sequencing of the amplified DNA comprising the plurality of target loci to obtain sequence reads, wherein an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA having the SNV locus is ~~detected~~ from the sequence reads.

determined to be present



NeoGenomics

Doc. 352-10 ('596 Patent), Claim 1.

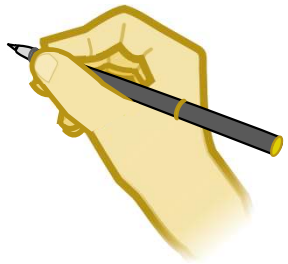
- NeoGenomics replaces "detected" with "determined to be present"

Doc. 372 at 15-16.

NeoGenomics Rewrites The Claim *Again* In Its Responsive Brief

the sequence reads are analyzed after sequencing to determine whether the SNV is present

performing high-throughput sequencing of the amplified DNA comprising the plurality of target loci to obtain sequence reads, wherein ~~an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA having the SNV locus is detected from the sequence reads~~.



NeoGenomics

Doc. 352-10 ('596 Patent), Claim 1.

- NeoGenomics replaces the entire "wherein" clause with an analytical step that occurs after sequencing to correct errors

Doc. 372 at 15-16.

NeoGenomics' Citation To Embodiments Is Misleading

Exemplary Embodiments for Detecting Single Nucleotide Variants

In certain aspects, provided herein are methods for detecting single nucleotide variants in a sample. The improved methods provided herein can achieve limits of detection of 0.015, 0.017, 0.02, 0.05, 0.1, 0.2, 0.3, 0.4 or 0.5 percent SNV in a sample. All the embodiments for detecting SNVs can be carried out with a system. The disclosure provides teachings regarding specific functional and structural features to carry out the methods. Furthermore, provided herein are embodiments comprising a nontransitory computer readable medium comprising computer readable code that, when executed by a processing device, causes the processing device to carry out the methods for detecting SNVs provided herein.

Accordingly, provided herein in one embodiment, is a method for determining whether a single nucleotide variant is present at a set of genomic positions in a sample from an individual, the method comprising:

a. for each genomic position, generating an estimate of efficiency and a per cycle error rate for an amplicon spanning that genomic position, using a training data set;
b. receiving observed nucleotide identity information for each genomic position in the sample;
c. determining a set of probabilities of single nucleotide variant percentage resulting from one or more real mutations at each genomic position, by comparing the observed nucleotide identity information at each genomic position to a model of different variant percentages using the estimated amplification efficiency and the per cycle error rate for each genomic position independently; and
d. determining the most-likely real variant percentage and confidence from the set of probabilities for each genomic position.

In illustrative embodiments of the method for determining whether a single nucleotide variant is present, the estimate of efficiency and the per cycle error rate is generated for a set of amplicons that span the genomic position. For example, 2, 3, 4, 5, 10, 15, 20, 25, 50, 100 or more amplicons can be included that span the genomic position.

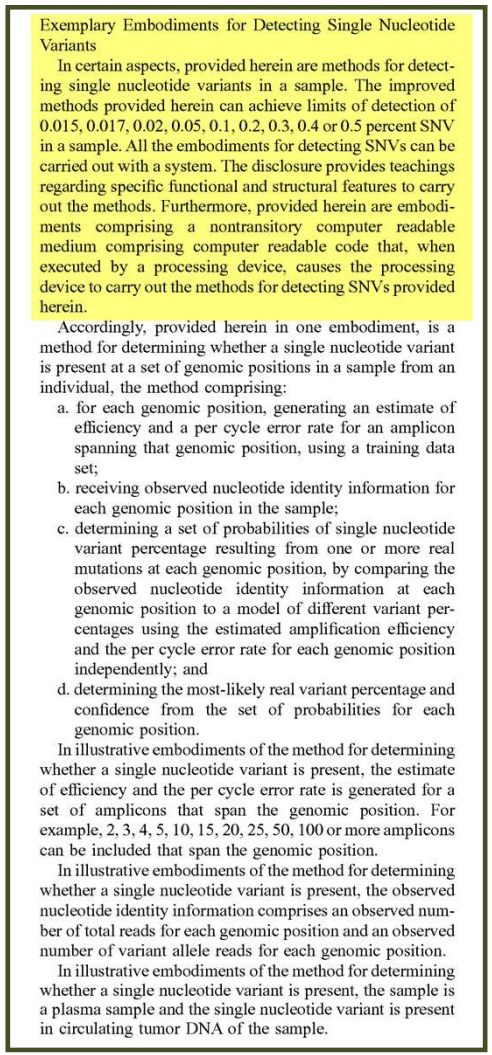
In illustrative embodiments of the method for determining whether a single nucleotide variant is present, the observed nucleotide identity information comprises an observed number of total reads for each genomic position and an observed number of variant allele reads for each genomic position.

In illustrative embodiments of the method for determining whether a single nucleotide variant is present, the sample is a plasma sample and the single nucleotide variant is present in circulating tumor DNA of the sample.

- Describes detecting SNVs generally
- Embodiments for downstream analysis of SNVs detected from the sequence reads
- Embodiment for sample source

Doc. 378 at 15;
Doc. 379 , ¶74.
Doc. 352-10 ('596 Patent) at 5:4-26; 65:14-67.

NeoGenomics' Citation To Embodiments Is Misleading



Exemplary Embodiments for Detecting Single Nucleotide Variants

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Accordingly, provided herein in one embodiment, is a method for determining whether a single nucleotide variant is present at a set of genomic positions in a sample from an individual, the method comprising:

a. for each genomic position, generating an estimate of efficiency and a per cycle error rate for an amplicon spanning that genomic position, using a training data set;
b. receiving observed nucleotide identity information for each genomic position in the sample;
c. determining a set of probabilities of single nucleotide variant percentage resulting from one or more real mutations at each genomic position, by comparing the observed nucleotide identity information at each genomic position to a model of different variant percentages using the estimated amplification efficiency and the per cycle error rate for each genomic position independently; and
d. determining the most-likely real variant percentage and confidence from the set of probabilities for each genomic position.

In illustrative embodiments of the method for determining whether a single nucleotide variant is present, the estimate of efficiency and the per cycle error rate is generated for a set of amplicons that span the genomic position. For example, 2, 3, 4, 5, 10, 15, 20, 25, 50, 100 or more amplicons can be included that span the genomic position.

In illustrative embodiments of the method for determining whether a single nucleotide variant is present, the observed nucleotide identity information comprises an observed number of total reads for each genomic position and an observed number of variant allele reads for each genomic position.

In illustrative embodiments of the method for determining whether a single nucleotide variant is present, the sample is a plasma sample and the single nucleotide variant is present in circulating tumor DNA of the sample.

- Describes detecting SNVs generally

Exemplary Embodiments for Detecting Single Nucleotide Variants

In certain aspects, provided herein are methods for detecting single nucleotide variants in a sample. The improved methods provided herein can achieve limits of detection of 0.015, 0.017, 0.02, 0.05, 0.1, 0.2, 0.3, 0.4 or 0.5 percent SNV in a sample. All the embodiments for detecting SNVs can be carried out with a system. The disclosure provides teachings regarding specific functional and structural features to carry out the methods. Furthermore, provided herein are embodiments comprising a nontransitory computer readable medium comprising computer readable code that, when executed by a processing device, causes the processing device to carry out the methods for detecting SNVs provided herein.

Doc. 378 at 15;
Doc. 379 , ¶74.
Doc. 352-10 ('596 Patent) at 5:4-26; 65:14-67.

NeoGenomics' Citation To Embodiments Is Misleading

Exemplary Embodiments for Detecting Single Nucleotide Variants

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Accordingly, provided herein in one embodiment, is a method for determining whether a single nucleotide variant is present at a set of genomic positions in a sample from an individual, the method comprising:

a. for each genomic position, generating an estimate of efficiency and a per cycle error rate for an amplicon spanning that genomic position, using a training data set;
b. receiving observed nucleotide identity information for each genomic position in the sample;
c. determining a set of probabilities of single nucleotide variant percentage resulting from one or more real mutations at each genomic position, by comparing the observed nucleotide identity information at each genomic position to a model of different variant percentages using the estimated amplification efficiency and the per cycle error rate for each genomic position independently; and
d. determining the most-likely real variant percentage and confidence from the set of probabilities for each genomic position.

In illustrative embodiments of the method for determining whether a single nucleotide variant is present, the estimate of efficiency and the per cycle error rate is generated for a set of amplicons that span the genomic position. For example, 2, 3, 4, 5, 10, 15, 20, 25, 50, 100 or more amplicons can be included that span the genomic position.

In illustrative embodiments of the method for determining whether a single nucleotide variant is present, the observed nucleotide identity information comprises an observed number of total reads for each genomic position and an observed number of variant allele reads for each genomic position.

In illustrative embodiments of the method for determining whether a single nucleotide variant is present, the sample is a plasma sample and the single nucleotide variant is present in circulating tumor DNA of the sample.

- Embodiments for downstream analysis of SNVs detected from the sequence reads

Accordingly, provided herein in one embodiment, is a method for determining whether a single nucleotide variant is present at a set of genomic positions in a sample from an individual, the method comprising:

a. for each genomic position, generating an estimate of efficiency and a per cycle error rate for an amplicon spanning that genomic position, using a training data set;
b. receiving observed nucleotide identity information for each genomic position in the sample;
c. determining a set of probabilities of single nucleotide variant percentage resulting from one or more real mutations at each genomic position, by comparing the observed nucleotide identity information at each genomic position to a model of different variant percentages using the estimated amplification efficiency and the per cycle error rate for each genomic position independently; and
d. determining the most-likely real variant percentage and confidence from the set of probabilities for each genomic position.

Doc. 378 at 15;
Doc. 379 , ¶74.
Doc. 352-10 ('596 Patent) at 5:4-26; 65:14-67.

NeoGenomics' Citation To Embodiments Is Misleading

Exemplary Embodiments for Detecting Single Nucleotide Variants

In certain aspects, provided herein are methods for detecting single nucleotide variants in a sample. The improved methods provided herein can achieve limits of detection of 0.015, 0.017, 0.02, 0.05, 0.1, 0.2, 0.3, 0.4 or 0.5 percent SNV in a sample. All the embodiments for detecting SNVs can be carried out with a system. The disclosure provides teachings regarding specific functional and structural features to carry out the methods. Furthermore, provided herein are embodiments comprising a nontransitory computer readable medium comprising computer readable code that, when executed by a processing device, causes the processing device to carry out the methods for detecting SNVs provided herein.

Accordingly, provided herein in one embodiment, is a method for determining whether a single nucleotide variant is present at a set of genomic positions in a sample from an individual, the method comprising:

a. for each genomic position, generating an estimate of efficiency and a per cycle error rate for an amplicon spanning that genomic position, using a training data set;
b. receiving observed nucleotide identity information for each genomic position in the sample;
c. determining a set of probabilities of single nucleotide variant percentage resulting from one or more real mutations at each genomic position, by comparing the observed nucleotide identity information at each genomic position to a model of different variant percentages using the estimated amplification efficiency and the per cycle error rate for each genomic position independently; and
d. determining the most-likely real variant percentage and confidence from the set of probabilities for each genomic position.

In illustrative embodiments of the method for determining whether a single nucleotide variant is present, the estimate of efficiency and the per cycle error rate is generated for a set of amplicons that span the genomic position. For example, 2, 3, 4, 5, 10, 15, 20, 25, 50, 100 or more amplicons can be included that span the genomic position.

In illustrative embodiments of the method for determining whether a single nucleotide variant is present, the observed nucleotide identity information comprises an observed number of total reads for each genomic position and an observed number of variant allele reads for each genomic position.

In illustrative embodiments of the method for determining whether a single nucleotide variant is present, the sample is a plasma sample and the single nucleotide variant is present in circulating tumor DNA of the sample.

> In illustrative embodiments of the method for determining whether a single nucleotide variant is present, the sample is a plasma sample and the single nucleotide variant is present in circulating tumor DNA of the sample.

- Embodiment for sample source

Doc. 378 at 15;
Doc. 379 , ¶74.
Doc. 352-10 ('596 Patent) at 5:4-26; 65:14-67.

"Sequence Read" Is Defined As Being Generated In A Sequencer

> Sequence Read refers to data representing a sequence of nucleotide bases that were measured, e.g., using a clonal sequencing method. Clonal sequencing may produce sequence data representing single, or clones, or clusters of one original DNA molecule. A sequence read may also have associated quality score at each base position of the sequence indicating the probability that nucleotide has been called correctly.

Doc. 352-10 ('596 Patent) at 31:16-24.

Doc. 378 at 14-15; Doc. 379, ¶¶ 46-47;
Doc. 154 at 25:20-26:5.

NeoGenomics Mischaracterizes What Is Required By Claim

What is claimed is:

1. A method for preparing biological samples useful for monitoring the progression of cancer in a subject, the method comprising:

...

(c) assaying cell-free DNA isolated from a plurality of biological samples obtained from the subject at different time points, wherein the assaying comprises:

performing targeted multiplex PCR amplification to amplify the plurality of target loci together in the same reaction volume from the isolated cell-free DNA using primers specific to the plurality of target loci for the individual subject; and

performing high-throughput sequencing of the amplified DNA comprising the plurality of target loci to obtain sequence reads, wherein an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA having the SNV locus is detected from the sequence reads.

Doc. 352-10 ('596 Patent), Claim 1.

- No recited limitations about a threshold for accuracy or errors
- Only requires detecting at least one SNV from a sequence read in ~ 7,000 reads

Doc. 378 at 16-17;
Doc. 379, ¶¶ 85-87.

Dr. Lennon Agrees: Claim Does Not Require Correctness of Calls

Dr. Niall Lennon
NeoGenomics' Expert

Q. Does Claim 1 require that a certain proportion of SNVs be called correctly?

A. I don't think Claim 1 has any wording around correctness.

Doc. 378 at 17; Doc. 378-4 at 32:12-15.

Dr. Metzker: Errors Do Not Negate That Sequencer Detects SNVs

Dr. Michael Metzker
Natera's Expert

Baylor College of Medicine

RICE

REDVAULT BIOSCIENCES

Lasergen

Q. Right. And so errors don't impede the ability to detect an SNV in your view, right?

A. Errors do not impede the ability to detect SNV mutations, that's correct. It becomes more challenging, and there are ways of handling that.

Doc. 392 at 9; Doc. 388-1 at 88: 2-17.

NeoGenomics Selectively Omits Dr. Metzker's Distinctions

Dr. Michael Metzker
Natera's Expert

Baylor College of Medicine
RICE
REDVAULT BIOSCIENCES
Lasergen

Q. …Can errors make it more difficult to detect SNV mutations?

A. Not in the context of the claim.

Q. How about in the context of Forshew?

A. In the real world, we know there are errors, and there are ways of weeding out the errors out of the sequencing data.

Doc. 388-1 at 102:11-103:4; Doc. 392 at 10.

NeoGenomics Mischaracterizes the Prosecution History

- NeoGenomics' assertion that "wherein" clause means "SNV mutation is determined to be present" based on file history is untrue.
- Natera distinguished *recited* claim language and quoted asserted reference:

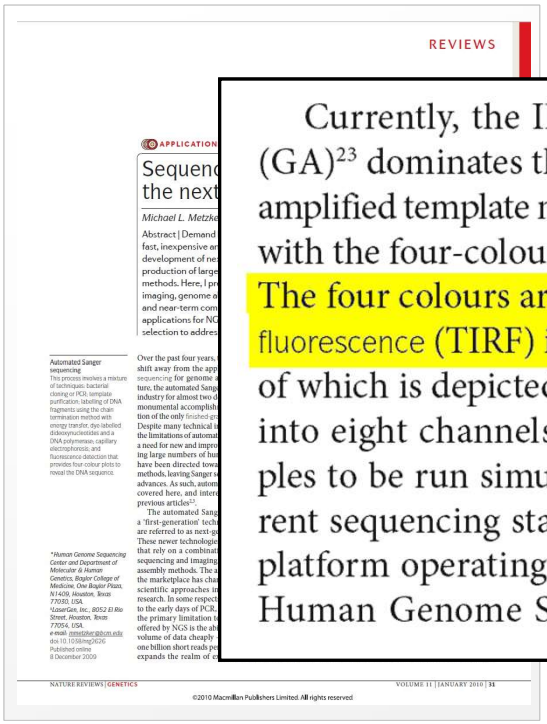
> In addition, Diehn also fails to teach or suggest "performing high-throughput sequencing of the amplified DNA comprising the plurality of target loci to obtain sequence reads, wherein an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA having the SNV locus is detected from the sequence reads." In contrast to the presently claimed invention, Diehn discloses "NSCLC DNA were accurately detected at fractional abundances between 0.025% and 10%" (paragraph 0792) and "fractions of tumor-derived DNA detected in plasma by SNV and/or indel reporters ranged from ~0.02% to 3.2%" (paragraph 0797). Further, Murtaza discloses detection of mutant allele frequency (AF) between 3% to 34% in patient plasma samples (Table 1).[116]

Doc. 374-9 at 7.

Doc. 373 at 17-18; Doc. 378 at 16; Doc. 379, ¶¶76-83.

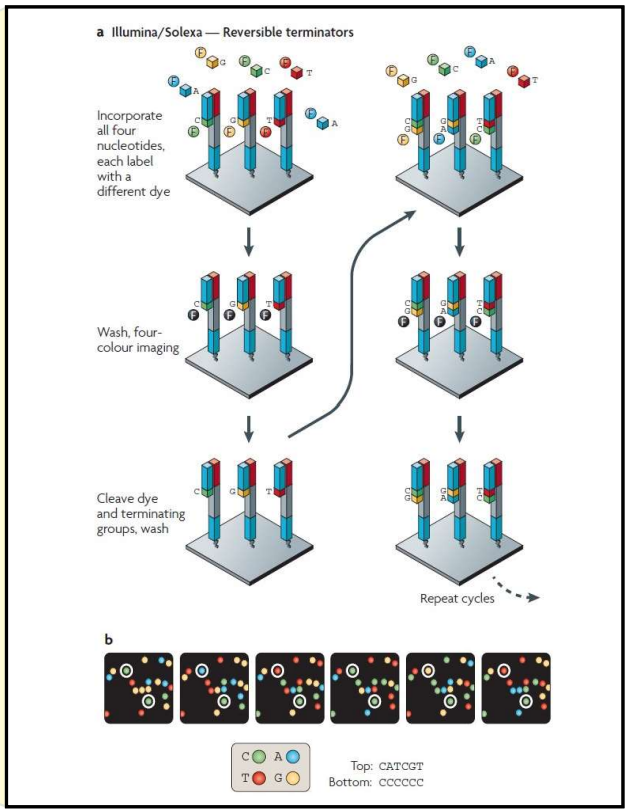
NeoGenomics Mischaracterizes Dr. Metzker's 2010 Review Article

- Assertion that "detection" refers to data processing is untrue.
- Article describes detection occurring in the sequencer:



Currently, the Illumina/Solexa Genome Analyzer (GA)[23] dominates the NGS market. It uses the clonally amplified template method illustrated in FIG. 1b, coupled with the four-colour CRT method illustrated in FIG. 2a. The four colours are detected by total internal reflection fluorescence (TIRF) imaging using two lasers, the output of which is depicted in FIG. 2b. The slide is partitioned into eight channels, which allows independent samples to be run simultaneously. TABLE 1 shows the current sequencing statistics of the Illumina/Solexa GA_{II} platform operating at the Baylor College of Medicine Human Genome Sequencing Center (BCM-HGSC;

Doc. 374-8 at 1, 5.



Doc. 374-8 at Fig. 2a, 2b.

NeoGenomics' Reliance On *Griffin* Is Misplaced

Griffin Claim Language:

A method for diagnosing an increased risk for thrombosis or a genetic defect causing thrombosis comprising the steps of:
(A) obtaining, from a test subject, test nucleic acid comprising codon 506 within EXON 10 of the human Factor V gene; and
(B) assaying for the presence of a point mutation in the nucleotides of codon 506 within EXON 10 of the human Factor V gene, ***wherein*** said point mutation correlates to a decrease in the degree of inactivation of human Factor V and/or human Factor Va by activated protein C, ***wherein*** the presence of said point mutation in said test nucleic acid indicates an increased risk for thrombosis or a genetic defect causing thrombosis.

Griffin v. Bertina, 285 F.3d 1029, 1031 (Fed. Cir. 2002).

Griffin court held:

- "wherein" clauses relate back to and clarify what is required
- Elaborate meaning of preamble

Here:

- "wherein" clause refers back to sequencing
- NeoGenomics erroneously equates preamble to "determining whether cancer is present"

Doc. 380 at 16-17.

NeoGenomics Improperly Relies On Claim 12 To Narrow Claim 1

What is claimed is:

1. A method for preparing biological samples useful for monitoring the progression of cancer in a subject, the method comprising:

(a) performing sequencing on a tumor biopsy sample of the subject to identify a plurality of tumor-specific mutations, wherein the tumor-specific mutations comprise one or more single nucleotide variant (SNV) mutations;

(b) evaluating results of the sequencing on the tumor biopsy sample to determine a plurality of target loci specific to the subject, wherein each target locus spans a tumor-specific mutation of the identified plurality of tumor-specific mutations; and

(c) assaying cell-free DNA isolated from a plurality of biological samples obtained from the subject at different time points, wherein the assaying comprises:

performing targeted multiplex PCR amplification to amplify the plurality of target loci together in the same reaction volume from the isolated cell-free DNA using primers specific to the plurality of target loci for the individual subject; and

performing high-throughput sequencing of the amplified DNA comprising the plurality of target loci to obtain sequence reads, wherein an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA having the SNV locus is detected from the sequence reads.

12. The method of claim 1, wherein the method further comprises detecting recurrence and/or metastases of the cancer from the tumor-specific mutations detected in the cell-free DNA.

- Claim 12:
  - Recites "*further* comprises"

Doc. 373 at 19;
Doc. 378 at 17-18;
Doc. 379, ¶75.

Dr. Lennon Agrees: Claim 12 Does Not Limit Scope Of Claim 1

Dr. Niall Lennon
NeoGenomics' Expert

Q. Okay. So for clarity of the record, when you considered the language of Claim 12, the language "further comprises" did not inform your opinion of what is encompassed by Claim 12; is that correct?

A. No. Right. So, again, it -- it informs my opinion to the extent of understanding the plain English meaning of "further comprises." And, to me, that means sort of "in addition it also includes."

Doc. 378 at 17-18; Doc. 378-4 at 37:9-38:4.

NeoGenomics Disregards Dr. Metzker's Explanations

Dr. Michael Metzker
Natera's Expert

Baylor College of Medicine | RICE | REDVAULT BIOSCIENCES | Lasergen

Q. I understand. You said that a bunch of times. I want to understand the difference between you and Dr. Lennon. So the thing that you are saying is incorrect about Dr. Lennon's construction is that he is saying some analysis needs to be done in order to make a determination about whether there is or isn't an SNV that is present, right?

A. Correct.

Q. Okay.

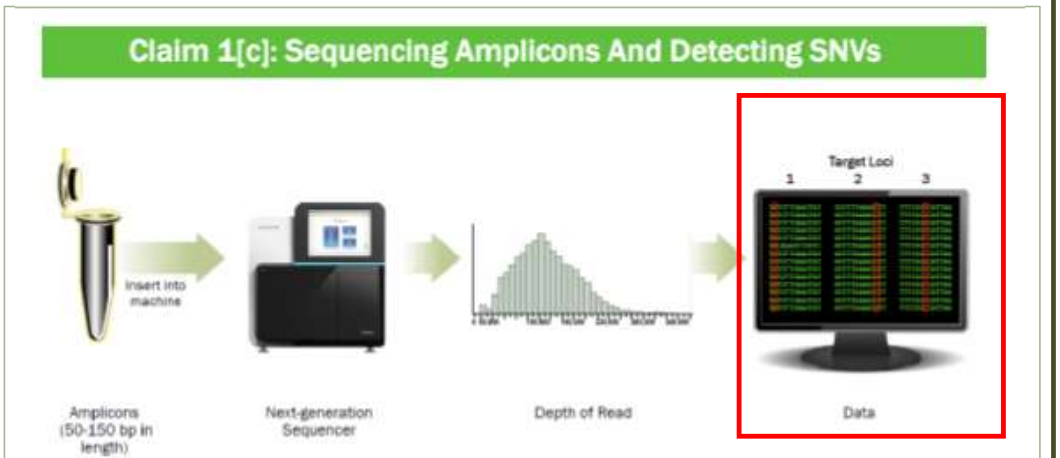
A. So my understanding is Dr. Lennon wants to rewrite the claim language, such as the detection or the determination of the presence of an SNV mutation happens downstream and outside of the sequencing process. I think he wants to rewrite the claim so that construction can hold, and I disagree with that.

Doc. 388-1 at 168:24-169:14; Doc. 392 at 3.

NeoGenomics Mischaracterizes Dr. Metzker's Demonstrative

NeoGenomics' Responsive Brief:

40, the data shown on slide 51 is divided into "Target Loci" 1, 2, and 3. Dkt. 246-1, 40, 51. This division represents the process of aligning the sequence reads to the target loci on the reference genome.



Doc. 380 at 14.

In Proper Context:

- Not an alignment to a reference genome
- Shows counting of reads generated for various SNV loci

B.3 "performing high-throughput sequencing of the amplified DNA"

'596 Patent, Claim 1

"performing high-throughput sequencing of the amplified DNA"

What is claimed is:

1. A method for preparing biological samples useful for monitoring the progression of cancer in a subject, the method comprising:

(a) performing sequencing on a tumor biopsy sample of the subject to identify a plurality of tumor-specific mutations, wherein the tumor-specific mutations comprise one or more single nucleotide variant (SNV) mutations;

(b) evaluating results of the sequencing on the tumor biopsy sample to determine a plurality of target loci specific to the subject, wherein each target locus spans a tumor-specific mutation of the identified plurality of tumor-specific mutations; and

(c) assaying cell-free DNA isolated from a plurality of biological samples obtained from the subject at different time points, wherein the assaying comprises:

performing targeted multiplex PCR amplification to amplify the plurality of target loci together in the same reaction volume from the isolated cell-free DNA using primers specific to the plurality of target loci for the individual subject; and

performing high-throughput sequencing of the amplified DNA comprising the plurality of target loci to obtain sequence reads, wherein an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA having the SNV locus is detected from the sequence reads.

Doc. 352-10 ('596 Patent), Claim 1.

"performing high-throughput sequencing of the amplified DNA"

Natera's Proposal	NeoGenomics' Proposal
Plain and ordinary meaning, which permits intermediate steps after multiplex amplification but before sequencing.	Plain and ordinary meaning, which is performing high-throughput sequencing of the amplified DNA obtained from the targeted multiplex PCR amplification step.

- Generating the sequence of the amplification products

- NeoGenomics inserts limitations that are not recited or suggested
- NeoGenomics ignores open-ended "comprising" language that permits intervening steps

Doc. 145 at ¶270; Doc. 372 at 17-23; Doc. 378 at 20-24.

Claim Terms Should Be Construed Consistently Across Patents

’454 Patent, Claim 1:

What is claimed is:

1. A method for preparing a plasma sample of a subject having cancer or suspected of having cancer useful for detecting one or more single nucleotide variant (SNV) mutations in the plasma sample, the method comprising:

performing whole exome sequencing or whole genome sequencing on a tumor sample of the subject to identify a plurality of tumor-specific SNV mutations;

performing targeted multiplex amplification to amplify 10 to 500 target loci each encompassing a different tumor-specific SNV mutation from cell-free DNA isolated from a plasma sample of the subject or DNA derived therefrom to obtain amplicons having a length of 50-150 bases, wherein the target loci are amplified together in the same reaction volume; and

sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads, wherein the sequencing has a depth of read of at least 50,000 per target locus.

Doc. 1-1 (’454 Patent), Claim 1.

’596 Patent, Claim 1:

What is claimed is:

1. A method for preparing biological samples useful for monitoring the progression of cancer in a subject, the method comprising:

...

(c) assaying cell-free DNA isolated from a plurality of biological samples obtained from the subject at different time points, wherein the assaying comprises:

performing targeted multiplex PCR amplification to amplify the plurality of target loci together in the same reaction volume from the isolated cell-free DNA using primers specific to the plurality of target loci for the individual subject; and

performing high-throughput sequencing of the amplified DNA comprising the plurality of target loci to obtain sequence reads, wherein an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA having the SNV locus is detected from the sequence reads.

Doc. 352-10 (’596 Patent), Claim 1.

NeoGenomics' Argument Premised On Nonexistent IPR Disclaimer

> Natera mischaracterizes the parties' disagreement over the "performing high-throughput sequencing of the amplified DNA" term as whether it "permits . . . intermediate steps after targeted multiplex amplification but before sequencing." Dkt. 372, 20. The dispute is much narrower and concerns whether Natera's disclaimer in the IPR on the '454 patent precludes ***intermediate PCR amplifications*** between targeted multiplex amplification and sequencing. That is how Natera distinguished its '454 patent claims over

Doc. 380 at 20-23.

The Court Already Rejected NeoGenomics' Analogous Argument

'454 Patent, Claim Construction Order:

First, Claim 1 is an open-ended, independent claim that "does not exclude additional, unrecited elements or method steps." *CollegeNet, Inc. v. ApplyYourself, Inc.*, 418 F.3d 1225, 1235 (Fed. Cir. 2005) (cleaned up). The claim should not be read to prohibit application of additional, unrecited steps to the amplicons before the amplicons are sequenced, so long as what is being sequenced are those amplicons.

Second, the rest of the '454 patent specification supports applying the plain and ordinary meaning. Claim 11 depends on Claim 1 and "comprises performing barcoding PCR prior to the sequencing." Doc. 1-1 at 222. "[I]f a dependent claim reads on a

Doc. 280.

Order Denying Reconsideration:

The Court has previously construed the term "sequencing the amplicons" in the '454 patent to have its plain and ordinary meaning. Doc. 280 at 15. This construction allows for intermediate steps such as barcoding between multiplex amplification and sequencing. Doc. 280 at 7-10. The defendant, Neogenomics Laboratories, Inc., asks the Court to modify this construction. It contends that the plaintiff, Natera, Inc., has taken a position before the Patent Trial and Appeal Board that is inconsistent with the construction it asked the Court to adopt and that the Court did adopt. Doc. 364. NeoGenomics takes the statements made by Natera out of context and interprets those statements in a way that is unfair and inaccurate. The motion will be denied.

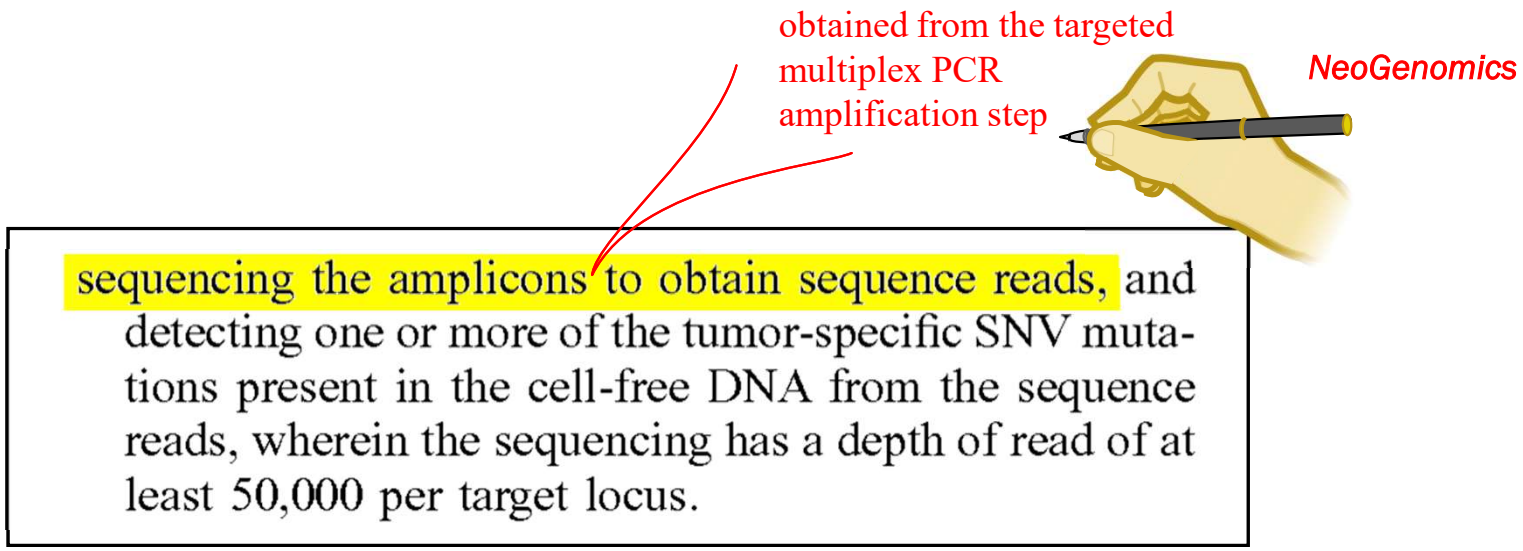
Doc. 385.

'596 Patent, Claim 1:

- Same open-ended "comprising" claim
- Same specification
- No disclaimer

Doc. 372 at 1, 18-20; Doc. 378 at 20-23.

NeoGenomics Rewrites The Claims



Doc. 352-10 ('596 Patent), Claim 1.

- NeoGenomics writes in language based on erroneous antecedent basis
- NeoGenomics' construction precludes attaching sequence adaptors and barcodes

Doc. 372 at 17-23; Doc. 378 at 20-24.

Barcoding PCR After Targeted Amplification, Before Sequencing

'596 Patent Specification:

> The amplified products were barcoded. One run of sequencing was performed with an approximately equal number of reads per sample.

Doc. 352-10 ('596 Patent) at 159:47-49.

> An aliquot of the STAR 2 products was then amplified by standard PCR for 12 cycles with 1 uM of tag-specific forward and barcoded reverse primers to generate barcoded sequencing libraries. An aliquot of each library was mixed with libraries of different barcodes and purified using a spin column.

Doc. 352-10 ('596 Patent) at 152:55-60.

Doc. 144 at 2; Doc. 372 at 20-21.

POSA Would Understand Sequencers Require Sequencing Tags

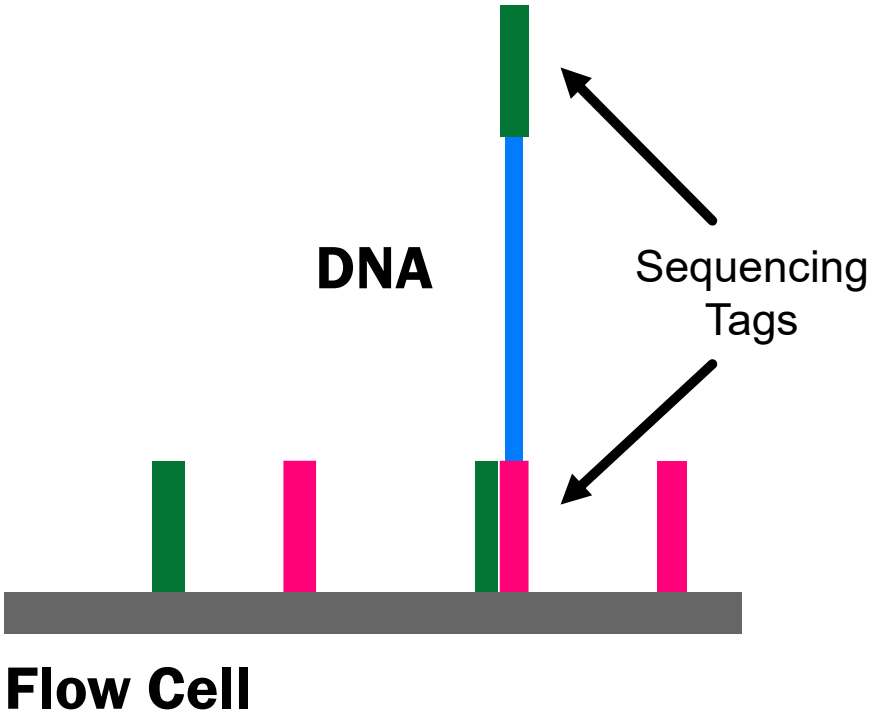
'596 Patent Specification:

> Full sequence tags and barcodes were attached to the amplification products and amplified for 9 cycles using adaptor specific primers. Prior to sequencing, the barcoded library product were pooled, purified with the QIAquick PCR Purification Kit (Qiagen), and quantified using the Qubit® dsDNA BR Assay Kit (Life Technologies). Amplicons were sequenced using an Illumina HiSeq 2500 sequencer.

Doc. 352-10 ('596 Patent) at 164:7-14.

Doc. 372 at 20; Doc. 145, ¶¶61, 274; Doc. 379, ¶¶90-94.

Sequencing Tags Attach Amplicons To Positions In Flow Cell



Doc. 372 at 20-22; Doc. 378 at 21-22;
Doc. 145 at ¶¶61,290; Doc. 379 at ¶¶26-37.

Dr. Van Ness Agrees Sequencing Adaptors Are Required For Illumina

Dr. Van Ness

Q. Next-generation sequencing generally requires adaptors to be present on amplified products for sequencing to occur, right? Sequencing adaptors?

A. That's true for Illumina. Not every platform requires adaptors.

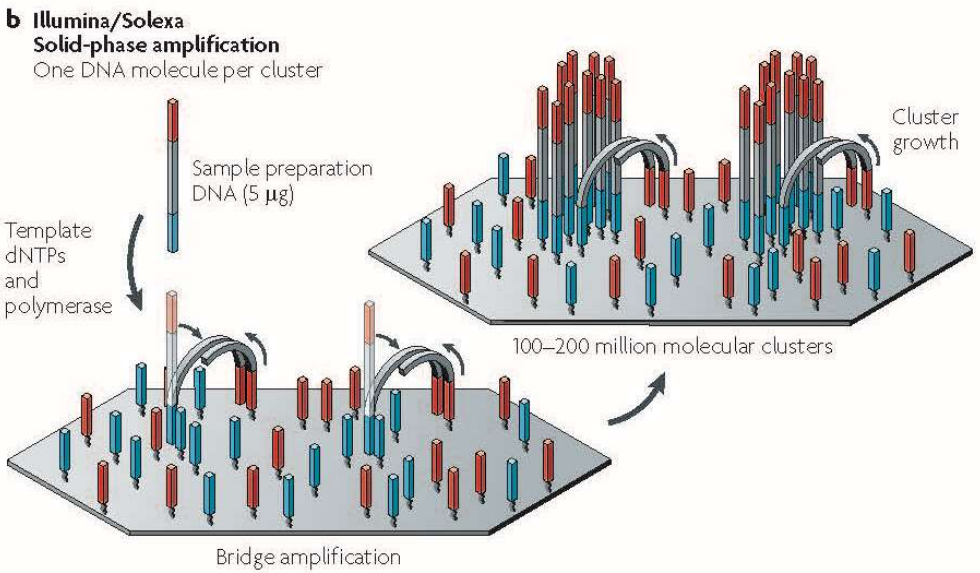
Q. But Illumina does?

A. But Illumina does.

Doc. 145-1 at ECF 61, 234:19-235:1.

NeoGenomics Reads Out All Examples Using Illumina Sequencers

- Illumina machines perform additional amplification before sequencing
- Bridge PCR occurs within sequencer before sequencing to increase signal



Doc. 145 at ¶¶61,290; Doc. 372 at 21;
Doc. 374-8 (Metzker 2010 Review Article) at Fig. 1b; Doc. 379 at ¶¶26-37.

Dr. Lennon: Composition Of Amplicons Does Not Necessarily Change

Dr. Niall Lennon
NeoGenomics' Expert

Q. Your testimony is that "It's a possibility that for some target loci, they do not receive a sequencing adapter in a subsequent amplification." And my question is: What happens to the other target loci?

A. So if there are target loci from the first reaction that are well-represented that you then put into a subsequent PCR amplification to add adapter sequences, for instance, they will get copied, amplified, and the amplicons that are created will have, you know, presumably the correct adapters appended to the tails.

Doc. 378 at 23-24; Doc. 378-4 at 52:20-53:8.